Journal of Applied Electrochemistry **33**: 647–650, 2003.

Technical note

Electrocoagulation and recovery of tannins from tree barks

S. CHOWWANAPOONPOHN[1], S. CHEWCHANWUTTIWONG[1], M.J. GARSON[2] and D. BUDDHASUKH[1,*]
[1]*Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, 50202, Thailand*
[2]*Department of Chemistry, School of Molecular & Microbial Sciences, The University of Queensland, Brisbane, QLD 4072, Australia*
*(*author for correspondence, fax: +66 53 892277, e-mail: duang@chiangmai.ac.th)*

Received 18 November 2002; accepted in revised form 11 February 2003

Key words: Castanopsis armata, electrocoagulation, electrolysis, *Lithocarpus elegans*, *Quercus brandisiana*, *Quercus kerrii*, *Quercus kingiana*

1. Introduction

Electrocoagulation, an electrochemical technique that has been used or is potentially useful in the treatment of a number of wastewaters, as well as in the production of potable water, involves the *in situ* formation of a metal hydroxide or metal ions by electrolysis. It is reported to be useful in removing ultrafine particles, various heavy metal ions, inorganic anions, colloidal suspensions, oil and grease, aquatic humus, certain synthetic dyes, as well as tannins and polyphenols in the industrial wastewaters [1–11]. However, there is almost no study on the recovery of substances removed by this technique [12]. There has been no study on the application of this method to recover natural phenolic compounds. The primary aim of this research is to investigate the potential of electrocoagulation technique as a method of recovering or enriching phenolic compounds from the crude aqueous solution of tree bark extractive. Previous phytochemical investigations from certain trees of *Lithocarpus*, *Quercus* and *Castonopsis* species revealed the presence of triterpenoids, steroids, flavonoids and polyphenolic principles, especially tannins as the major components in their barks [13–18]. Thus, five uninvestigated members from these genus, namely *Lithocarpus elegans*, *Quercus kerrii*, *Quercus brandisiana*, *Quercus kingiana* and *Castanopsis armata* were selected for study. Our previous work using simple phenolic compounds as well as commercial tannin indicated that some of these substances can be recovered unchanged from the coagulum resulting from electrolysis of their aqueous solutions [12]. The recovery method involves dissolving the coagulum in a dilute acid solution and extracting with an alcohol (1-butanol). In the following study, we report similar operations performed on aqueous extracts of barks from the five tree species mentioned above. Since polyphenolic substances usually exhibit a certain degree of antioxidising property, this was also determined on the recovered extractives.

2. Materials and methods

2.1. *Chemicals*

Myoglobin and ABTS (2,2-Azino-bis(3-ethylbenthoazoline-6-sulfonic acid) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Trolox (6-hydroxy-2,5,7,8-tetramethylchlorman-2-carboxylic acid) were purchased from Aldrich Chemical Co. (St. Louis, MO, USA). Hydrogen peroxide 30% was purchased from Carlo Erba Reagent Co. (Ronando, MI, Italy). Sodium tungstate ($Na_2WO_4 \cdot 2H_2O$), sodium carbonate (Na_2CO_3) and tannic acid were purchased from May & Bayer Ltd (Manchester, UK). Hydrochloric acid was purchased from Merck KGa A (Darmstadt, Germany). Phosphomolybdic acid was purchased from Prolabo (Paris, France). Phosphoric acid was purchased from BDH Laboratory Supplies (Dorset, England). Butan-1-ol was used as analytical grade.

2.2. *Plant material*

The stem barks of *Lithocarpus elegans*, *Quercus kerrii*, *Quercus brandisiana*, *Quercus kingiana* and *Castanopsis armata* were collected from the Doi Suthep-Pui National Park, east side of Doi Suthep at Pah Lad Temple, Chiang Mai, Thailand, on 16 June 2002. The plant materials were identified by J. Maxwell from the Department of Biology at Chiang Mai University. Voucher specimens were deposited at the Department of Biology Herbarium, Chiang Mai University with the accession numbers S-1, S-3(S-10), S-11, S-13 and S-14, respectively.

2.3. *Electrocoagulation and recovery of polyphenolic compounds*

The bark of each of the five trees above was subjected to treatment outlined in Figure 1. The dried ground stem

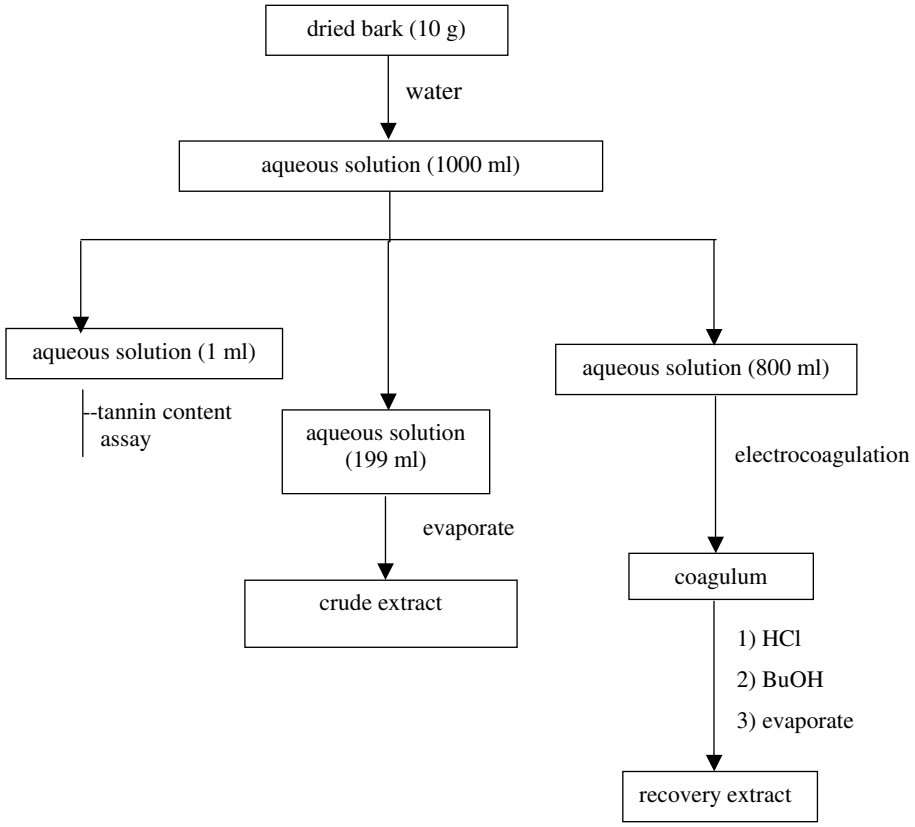

Fig. 1. Scheme of bark treatment.

bark (10 g) was extracted with water (1000 ml). After heating at 90 °C for 1 h, each afforded a coloured solution which was adjusted to 1000 ml, divided into three portions each of which was treated as follows:

(a) Portion 1 (1 ml) was analysed for tannin content using Folin–Denis reagent [19].
(b) Portion 2 (199 ml) was directly evaporated and dried under vacuum to afford the unelectrocoagulated crude extract as a deep red solid substance. Determinations of tannin content and antioxidant activity were performed on this crude extract.
(c) Portion 3 (800 ml) was placed in glass tank (dia. 11 cm, height 23 cm). Two aluminium plates (dimension 30 cm × 10 cm × 0.05 cm) were used as electrodes. These were dipped 3 cm apart and 9 cm deep into the magnetically-stirred solution. Sodium chloride (1.6 g) was added as a supporting electrolyte. Direct current (2.0 A, 16 V) from a d.c. power supplier was then passed through the solution via the two electrodes for 1.5 h. The resulting mixture was filtered through a Buchner funnel. The precipitate (coagulum) was collected and left for 24 h to dry at room temperature, then dissolved in 8% hydrochloric acid solution. The obtained acidic solution was extracted with 1-butanol [12]. Evaporation of the alcoholic solution afforded the recovery extract, which was taken for determination of tannin content and antioxidant activity.

2.4. *Determination of tannin*

Tannin was determined by complexing with Folin–Denis reagent [19]. The resulting complex was taken for absorbance measurement at 760 nm and quantitated using standard tannic acid calibration curve.

2.5. *Determination of antioxidant activity*

The method was modified from that reported by Miller et al. [20]. Metmyoglobin was purified by adding the stock myogloblin solution (400 μmol l^{-1}) in 0.15 mol l^{-1} isotonic phosphate buffer saline (PBS), pH 7.4, to an equal volume of freshly prepared (740 μmol l^{-1}) potassium ferricyanide solution. After mixing, the solution was passed through a G15-120 Sephadex column equilibrated in the buffer, and the metmyoglobin fraction was collected. The final concentration of purified metmyoglobin was estimated by applying the Whiteburn equations [20]: $[\text{Met Mb}] = 146A_{490}–108A_{560} + 2.1A_{580}$; $[\text{Ferryl Mb}] = -62A_{490} + 242A_{560} - 123A_{580}$ $[\text{MbO2}] = 2.8A_{490} - 127A_{560} + 153A_{580}$ where Mb is myogloblin. These equations are derived by solving simultaneous equation based on Beer's law, measuring the absorbance at 490, 560 and 580 nm and subtracting the reading at 700 nm to correct for background absorbance. Normally the metmyogloblin fraction is >95% of the total haem protein. A 2.5 mmol Trolox solution was prepared by dissolving 0.1610 g of Trolox

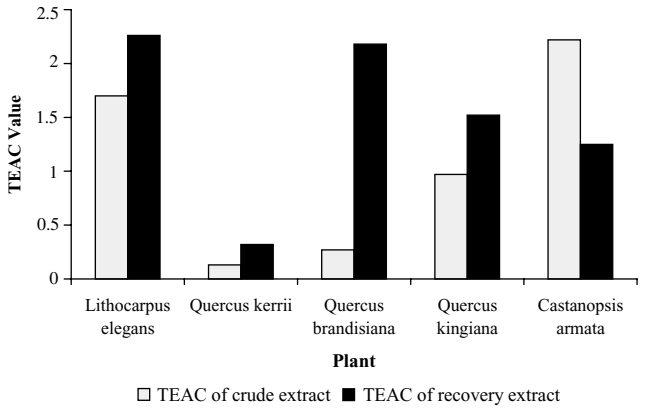

Fig. 2. Antioxidant activities of crude tree bark extracts against recovery extract. (Trolox equivalent antioxidant capacity (TEAC), defined as per Table 3.)

in 250 ml of buffer. ABTS was prepared as a 5 mmol l^{-1} solution by dissolving 0.02799 g in 10.0 ml of buffer; 500 μmol l^{-1} ABTS was prepared from this stock solution for use as a working reagent. Hydrogen peroxide was diluted to a 0.45 mmol l^{-1} working solution.

2.6. *Spectroscopic antioxidant assay*

ABTS (500 μl, 500 μmol l^{-1}), 70 μl of metmyogloblin (76 μmol l^{-1}) and 1000 μl of buffer (of which 20 μl from an appropriate dilution was replaced when a sample was being investigated) were mixed, and the reaction was initiated by the addition of 450 μl of hydrogen peroxide (500 μmol l^{-1}). The reaction was recorded on time course program of UV-2410 PC spectrophotometer for 180 s at 414 nm and determined in duplication. The reaction rates were used for calculation of the antioxidant activity (Figure 2).

3. Results and discussion

The amount of the recovery extract for each plant, obtained by extracting the acid solution of the coagulum with 1-butanol, is shown in Table 1. This amount is between 59–95% of the crude extract. Table 2 shows the contents of phenolic substances in the form of tannin in the bark, the crude extract, and the recovery extract of each of the plants studied. From these results, an enrichment of between 0–48% of the phenolic substances in the recovery extract as compared to the crude extract is indicated.

From Figure 2 and Table 3, it is evident that both the crude extracts and the recovery extracts all have a certain degree of antioxidant activity, presumably due mainly to the phenolic substances in the extracts. However, all the recovery extracts, except one, clearly exhibit more activity than the crude extracts. This is understandable in view of the phenolic (tannin) enrichment obtained in the recovery extracts, although the correlation between the degree of tannin enrichment and antioxidant activity increase is rather poor. In case of

Table 1. Amounts of tree bark extracts before and after electrocoagulation

Plant	Crude extract (based on dried bark) /%	Recovery extract (based on dried bark) /%	Recovery extract (based on crude extract) /%
Lithocarpus elegans	21.9	16.8	77.0
Quercus kerrii	16.1	15.3	95.7
Quercus brandisiana	15.0	13.1	87.5
Quercus kingiana	17.1	10.6	62.0
Castanopsis armata	16.2	9.6	59.2

Castanopsis armata, the recovery extract of which contained equal amount of tannin as the crude extract (73%), the antioxidant activity of the former even decreased 44% compared with that of the latter.

Recent studies have shown that electrocoagulation can be used to remove certain phenolic compounds including tannins [12], certain flavonoids [21], starch, and proteins [21] from an aqueous solution. Assuming the presence of starch and proteins are negligible in the barks selected for study in this research, the major composition of the electrolytic coagulum, and hence of the recovery extract obtained from it in this study must necessarily be phenolic substances, especially those with the 1,2-dihydroxy and 1,2,3-trihydroxy types [12, 21]. Tannins usually contain these groupings, as do many, but not all, members of the other classes of natural phenolic compounds. On the other hand functional groupings responsible for strong antioxidant activity seem to be more varied.

In this light, the seeming experimental discrepancy mentioned above, that is, the poor correlation between the degree of tannin enrichment and the increase (or even decrease) in antioxidant activity, is somewhat understandable or even expected. Apart from tannins, other phenolic substances with strong antioxidant activities, but which are not readily coagulated, might be present in the bark extracts. Moreover different tannins and their different degradation products might also show different degrees of antioxidant activity, although under the above-mentioned electrolytic conditions, the phenolic compounds (including tannins) are not expected to preferentially undergo degradation by anodic oxidation [12].

4. Conclusion

Electrocoagulation, a technique that is normally used for eliminating certain unwanted particles or chemical species from an aqueous solution, may also be of potential use in selectively coagulating certain desired organic substances from solution. In this study, phenolic substances from selected tree barks were coagulated electrolytically and subsequently recovered as concentrated phenolic extracts with overall increase in antioxidant activity.

Table 2. Tannin contents in tree barks, crude extracts and recovery extracts after electrocoagulation treatment

Plant	Tannins			Tannin enrichment based on crude extract /%
	Dried bark /%	Crude extract /%	Recovery extract /%	
Lithocarpus elegans	6.7	30.6	44.1	44
Quercus kerrii	6.1	38.4	52.1	35
Quercus brandisiana	9.1	61.1	88.4	44
Quercus kingiana	11.3	66.1	98.2	48
Castanopsis armata	11.9	73.6	73.4	0

Table 3. Antioxidant activity assay (ABTS/H_2O_2/metmyoglobin methods) of crude aqueous extract and recovery electrocoagulated extracts of certain tree barks

Sample	Absorbance of $ABTS^{\bullet+}$ radical cation at 180 s	Weight of sample /mg	$ABTS^{\bullet+}$ inhibition* /%	TEAC† /(mg trolox) (mg sample)$^{-1}$
Blank control	0.8350			
Lithocarpus elegans crude extract	0.4568	0.004	44.33	1.70
Lithocarpus elegans recovery extract	0.2877	0.0044	64.94	2.26
Quercus kerrii crude extract	0.7757	0.0064	5.46	0.13
Quercus kerrii recovery extract	0.7300	0.0052	11.03	0.32
Quercus brandisiana crude extract	0.7453	0.0052	9.17	0.27
Quercus brandisiana recovery extract	0.1655	0.0056	79.83	2.18
Quercus kingiana crude extract	0.4717	0.0070	44.47	0.97
Quercus kingiana recovery extract	0.4268	0.0050	49.75	1.52
Castanopsis armata crude extract	0.3578	0.0040	57.88	2.22
Castanopsis armata recovery extract	0.5730	0.0040	32.54	1.25

* $ABTS^{\bullet+}$ inhibition $= A_0 - A_{180}/A_0 \times 100$ where A_0 = absorbance blank control and A_{180} = absorbance of sample at 180 s.

† TEAC ≡ Trolox equivalent antioxidant capacity calibrated from the percentatge $ABTS^{\bullet+}$ inhibition curve of standard Trolox. The $ABTS^{\bullet+}$ radical cation absorbance of 0.00, 0.75, 1.25, 1.5, 2.0, 2.25 mM Trolox at 180 s was measured and converted to percentage $ABTS^{\bullet+}$ inhibition. Standard calibration curve of TEAC was plotted between percentage $ABTS^{\bullet+}$ trolox inhibition *vs* concentration to obtain the linear equation; $Y = 6.5284\ X$, $R^2 = 0.97$, where x = concentration of std. Trolox and Y = % $ABTS^{\bullet+}$ inhibition of Trolox. The scavenging of the $ABTS^{\bullet+}$ radical cation by crude and recovery electrocoagulated extract are determined relative to that of Trolox as an antioxidant standard. 2.50, 5.00, 7.50 μg Trolox have percentage inhibition 13.34, 26.09 and 39.44, respectively.

Acknowledgements

The authors are grateful for the financial support of the Royal Golden Jubilee Grant award from The Thailand Research Fund (to S.C.) and support by the University of Queensland are gratefully acknowledged. We thank Prof. Maitree Suttajit and Mr Nopporn Chiya for their assistance in ABTS antioxidant testing at Faculty of Medicine, Chiang Mai University. We also acknowledge Mr J.F. Maxwell for his plant identification.

References

1. M.J. Matterson, R.L. Dobson, R.W. Glenn Jr, N.S. Kukunoor, W.H. Waits III and E.J. Clayfield, *Colloids Surf. A: Physicochem. Eng. Aspects* **104** (1995) 101.
2. C.T. Tsai, S.T. Lin, Y.C. Shue and P.L. Su, *Water Res.* **31** (1997) 3073.
3. B.M. Belongia, P.D. Haworth, J.C. Baygent and S. Raghavan, *J. Electrochem. Soc.* **146** (1999) 4124.
4. M.F. Pouet and A. Grasmick, *Water Sci. Tech.* **31** (1995) 275.
5. N. Kongscricharoern and C. Polprasert, *Water Sci. Tech.* **31** (1995) 109.
6. X. Chen, G. Chen and P.L. Yue, *Sep. Purif. Technol.* **19** (2000) 65.
7. J. Mrozowski and J. Zielinski, *Environ. Protection Eng.* **9** (1983) 79.
8. J.S. Do and M.L. Chen, *J. Appl. Electrochem.* **24** (1994) 785.
9. A. Buso, L. Balbo, M. Giomo, G. Farnia and G. Sandona, *Ind. Eng. Chem. Res.* **39** (2000) 494.
10. N. Mameri, A.R. Yeddou, H. Lounici, D. Belhocine, H. Grib and B. Bariou, *Water Res.* **32** (1998) 1604.
11. V.E. Cenkin and A.N. Belevstev, *Effluent & Water Treatment J.* **25**(7) (1985) 243.
12. W. Phutdhawong, S. Chowwanapoonpohn and D. Buddhasukh, *Anal. Sci.* **16** (2000) 1083.
13. J.B. Harborne, H. Baxter and P.G. Moss, 'Phytochemical Dictionary: A Handbook of Bioactive Compounds from plants', 2nd edn (Taylor & Francis, London, 1999).
14. H.R. Arthur and P.D.S. Ko, *Phytochem.* **13** (1974) 2551.
15. W.H. Hui, P.D.S. Ko, Y.C. Lee, M.M. Li and H.R. Arthur, *Phytochem.* **14** (1975) 1063.
16. D. Sun, H. Wong and L.Y. Foo, *Phytochem.* **26** (1987) 1825.
17. Z.A. Kuliev, A.D. Vdovin, N.D. Abdullaev, A.B. Makhmatkulov and V.M. Malikov, *Chem. Nat. Comp.* **33** (1997) 642.
18. S. Ohara and R.W. Hemingway, *Holzforschung* **43** (1989) 149.
19. D.H. Dyer, 'The Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC)', Vol. 2 (15th edn) (AOAC Publishing, Virginia, 1990), p. 703.
20. N.J. Miller, C. Rice-Evans, M.J. Davies, V. Gopinathan and A. Milner, *Clin. Sci.* **84** (1993) 407.
21. S. Chowwanapoonpohn and D. Buddhasukh, *ACGC Chem. Res. Commun.* **14** (2002) 70.